REPRODUCTIVE BEHAVIOR OF THE POTATO TUBERWORM, *PHTHORIMAEA OPERCULELLA* (ZELLER), (LEPIDOPTERA: GELECHIIDAE) AND THE EFFECT OF THE CHEMOSTERILANT METEPA

By

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INTRODUCTION

The potato tuberworm, Phthorimaea operculella (Zeller), is a widely distributed pest of potatoes, Solanum tuberosum Linnaeus, injuring the growing plant in the field and, more importantly, infesting tubers in storage. Other plants attacked by this species include tobacco, Nicotiana tabacum Linnaeus (the insect is called the tobacco splitworm on this host); tomatoes, Lycopersicum esculentum Linnaeus; peppers, Capsicum frutescens Linnaeus; and a number of uncultivated plants in the family Solanaceae.

The tuberworm is an important pest on the island of Bermuda. The small area and extreme isolation of Bermuda, the comparatively limited host range of P. operculella, and the local seasonal fluctuations in the population of this species encouraged me to investigate the possibility of instituting some form of eradication program against the pest on the island.

Knipling (1955, 1959, and 1964) discussed the potential role of the sterility method for controlling insect populations. This method was initially limited to the release of radiation-sterilized insects into the natural population, but has recently been broadened by the discovery of a number of chemicals capable of inducing sterility in insects (LaBrecque, 1961; Smith, LaBrecque and Borkovec, 1964).

Eradication of the potato tuberworm from Bermuda through a program of chemosterilization appeared possible, particularly in light of the recent success in sterilization of the pink bollworm, Pectinophora gossypiella (Saunders), a member of the same family as the tuberworm (Ouye, Garcia, and Martin, 1965).

Before a decision could be made on the feasibility of such a program, basic information was needed on (i) the morphology of the male and female reproductive systems of the potato tuberworm moth, (ii) the reproductive behavior of the insect, including the importance of sex pheromones in this behavior, and (iii) the effectiveness of a candidate chemosterilant, in this case metepa (tris[1-(2-methyl) aziridinyl]phosphine oxide) against the potato tuberworm. Research in these three areas is the basis for this dissertation.

LITERATURE REVIEW

History and Biology of the Potato Tuberworm

Distribution

The first record of the potato tuberworm was by Berthon (1855) who described a "grub" damaging potatoes in Tasmania in 1854 and suggested that this was the same pest which had in previous years caused damage to potatoes in New Zealand. The insect was reported from California in 1856, Texas in 1862, southern Europe in 1874 (established some years earlier), and Florida and Virginia in 1897 (Graf, 1917). The virtual simultaneous discovery of the pest in California, New Zealand, and in southern Europe led to controversy over the origin of *P. operculella*. McCarty (1897) believed that the species originated in the United States and gave as his reasons the endemic occurrence of potato and tobacco in this country, the early report of natural enemies of the tuberworm in the southern United States, and the severe damage caused by this pest in countries other than the United States.

Botanists now consider the potato to have originated in the elevated valleys of the Andes mountains of South America (Correll, 1962), and tobacco in Central America (Hill, 1952), so perhaps one of these areas might more reasonably be considered as the center of dispersal of the tuberworm.

The tuberworm has been very successful in spreading into a great many tropical, subtropical, and temperate regions of the world (Commonwealth Institute of Entomology, 1951). Transport of infested potato tubers appears to have been responsible for most of the long-range dispersal of the species. Berthon (1855) suggested that establishment of the tuberworm in Tasmania was the result of the importation of infested tubers from New Zealand. One hundred years later, this method of dispersal was still successful, as evidenced by the introduction of the pest into Japan in imported potatoes (Koizumi, 1955). Introduction of P. operculella into Bermuda in 1931 must certainly have been the result of this passive form of dispersal (Department of Agriculture, Bermuda, unpublished data).

Appearance, life history, and damage

Adult tuberworms are small, grey moths with dark brown and black mottling, having narrow wings spanning approximately 16 mm. Eggs are 0.4 to 0.5 mm long and approximately 0.35 mm wide. When freshly laid they are pearly white in color, and become light brown at the time of hatching (Graf, 1917). Newly emerged larvae are 1 to 2 mm long; they are light yellow with a dark brown head capsule. The color of mature larvae is variable, often pink, but occasionally pale green when entering the prepupal stage, at which time the larvae are 10 to 14 mm long. Pupae are initially white with light green markings, but become brown at maturity (Hofmaster, 1949).

Details on the life history of the potato tuberworm have been given by Picard (1913a), Graf (1917), Poos and Peters (1927), Hofmaster (1949), and Koizumi (1955). A life cycle can be completed in 17 days at 35 C but requires 209 days at 10 C (Koizumi, 1955). At 26.5 C the egg stage lasts 5 days, the four larval stages 10 to 11 days, and the prepupal and pupal stages 12 days; a cycle is completed in 27 to 28 days (Finney, Flanders, and Smith, 1947).

Field infestations normally begin with eggs being laid on the undersides of potato leaves and the resulting larvae causing blotch mines in foliage. Eggs of subsequent generations are generally laid on exposed tubers, and in this manner the pest becomes established in storage. Infested tubers are riddled with winding tunnels filled with brown frass (Graf, 1917).

Fecundity

Picard (1913a) reported 50 to 80 eggs as the mean number produced by unfed tuberworm females and comparable figures have been given by Hofmaster (1949), Bartoloni (1951), and Koizumi (1955). Finney et al. (1947) indicated that a single female tuberworm moth would lay from 80 to 200 eggs when provided with nourishment. Labeyrie (1957a), investigating the influence of feeding on fecundity in the tuberworm, reported a mean of 84 eggs for unfed females and 236 for fed females. Fertility among tuberworms is generally very high, egg hatch normally exceeding 95 per cent (Koizumi, 1955). Unmated females consistently lay fewer eggs than those which have mated (Hofmaster, 1949).

Parthenogenetic reproduction

Picard (1913b), Poos and Peters (1927), and Hofmaster (1949) reported parthenogenetic reproduction in the potato tuberworm with offspring of both sexes resulting. In no case did the number of females reproducing in this manner exceed 10 per cent of the sample tested (Hofmaster, 1949). Cockayne (1935), and Kerr (1962) have discussed parthenogenesis and sex determination in the Lepidoptera.

Ratio of sexes

Graf (1917), and Poos and Peters (1927) considered the proportion of the tuberworm sexes to be equal, although the data given by Graf to support this belief does not meet the Chi-square test for 1:1 ratio at the 5 per cent level of probability, the males being more numerous than the females. Finney (personal communication, 1966) has stated that the ratio of the sexes among the many millions of tuberworms which he and his associates reared during mass production of Macrocentrus ancylivorus (Finney et al., 1947) was very close to 1:1.

Mass rearing of P. operculella

The potato tuberworm has been used successfully as a host for a number of entomophagous insects reared under laboratory conditions (Flanders, 1949). Finney et al. (1947) gave a detailed account of procedures used in mass culture of tuberworms. Marvin (1944) devised a simple method for obtaining a constant supply of eggs of P. operculella for use in rearing programs and Labeyrie (1957b) suggested modifica-

tions of the rearing procedure devised by Finney et al., which gave improved efficiency and better control of diseases in the cultures. The problem of disease among tuberworms is of particular importance when the insect is reared in large numbers, with bacteria (Steinhaus, 1945), protozoa (Allen and Brunson, 1947), and viruses (Labeyrie, 1957b), all capable of causing serious losses in cultures.

Reproduction in the Lepidoptera

Information on the morphology of the reproductive system and on reproductive behavior in Lepidoptera is available in papers by Cholodkovsky (1884), Stitz (1900, 1901), Ruckes (1919), Norris (1932), Musgrave (1937), Williams (1939, 1940), and more recently by Callahan (1958), Callahan and Chapin (1960), Callahan and Cascio (1963), and Tedders and Calcote (1967). Poos and Peters (1927) illustrated the external genitalia of male and female potato tuberworm moths, and Wellso and Adkinson (1962) illustrated and discussed the morphology of the female reproductive system of the gelechiid Pectinophora gossypiella, the pink bollworm of cotton.

The male reproductive system

Cholodkovsky (1884) recognized four classes of Lepidoptera based on testes types: (A) testes completely separate and four-lobed, as in the genus Hepialus; (B) testes separate but rounded and three-lobed, as in Saturnia; (C) discernibly separate testes enclosed in a single scrotum, as in the genus Lycaena; and (D) testes fused and appearing as a single round organ in a common scrotum.

Paired vas deferens extend from the base of the testes. Each vas deferens becomes dilated near the middle of its length to form the seminal vesicles in which sperm is stored. The vas deferens open midway on the branches of the ductus ejaculatorius duplex, a paired glandular reservoir for accessory gland substances and sperm bundles (Callahan and Chapin, 1960). A pair of tubular accessory glands arise anteriorly on the ductus ejaculatorius duplex (Norris, 1932). Caudaly the ductus ejaculatorius duplex unites to form the long ductus ejaculatorius simplex which has in turn been subdivided into two areas by Callahan (1958), the cephalad area designated as the primary segment, and the caudad region as the cuticular segment. The aedeagus, enclosing the eversible endophallus, terminates the male reproductive system (Norris, 1932).

The female reproductive system

A pair of ovaries, each (with but few exceptions) consisting of four polytrophic ovarioles, dominate the female reproductive system. The ovarioles of each ovarium fuse distally to a single unit but no common suspensory ligament appears to exist (Norris, 1932; Callahan, 1958). Eidman (1931) classified the Lepidoptera into three groups on the basis of the degree of development of ovaries at eclosion of the adult.

The two ovaries open into lateral oviducts which then join to form the common oviduct terminating with the ovipositor. A spermatheca and a pair of accessory glands

connect with the dorsal surface of the common oviduct (Norris, 1932). The bursa copulatrix, which receives the sperm during mating, is of particular interest in the Lepidoptera. In some groups this organ shares a single external opening with the oviduct, but more commonly the bursa copulatrix opens independently on the cuticula between the seventh and eighth abdominal sternites, and in such cases it is connected to the common oviduct by the ductus seminalis (Norris, 1932). Spermatophore placement within the bursa copulatrix has been studied in detail in the Noctuidae by Callahan (1961), and the relationship between the neck of the spermatophore and the ductus seminalis within the bursa copulatrix has been used by Williams (1941) as a basis for grouping Lepidoptera. Callahan and Cascio (1963) traced the path of sperm in the corn earworm from the spermatophore, through the ductus seminalis, across the common oviduct and up into the spermatheca.

The spermatophore

In the Lepidoptera sperm are transferred from the male to the female in a cuticularized spermatophore formed within the ductus ejaculatorius simplex of the male from secretions of this duct (Callahan, 1958). The shape of the spermatophore differs widely among species, even within a family (Williams, 1941), and the mechanism of spermatophore formation and transfer is often highly complex (Callahan, 1958). The presence of spermatophores within the bursa copulatrix of females is an accepted and convenient measure

of successful matings in Lepidoptera (Williams, 1938; Callahan, 1958; Ouye, Garcia, Graham, and Martin, 1965).

Sex attractants in Lepidoptera

Sex attractants, released from glands in the terminal abdominal segments (Götz, 1951; Jefferson, Shorey, and Gaston, 1966), are important in mating behavior in more than 100 species of Lepidoptera (Jacobson, 1965). Although pheromones released by females frequently attract males from a distance, they may simply serve to excite males which are nearby (Jacobson, 1965). Bossert and Wilson (1963) have discussed olfactory communication among animals, and have established methods for determining parameters of effectiveness of olfactory systems.

The success in isolating, chemically characterizing, and synthesizing sex attractants from the gypsy moth, Porthetria dispar (Linnaeus), and the silkworm, Bombyx mori (Linnaeus), has encouraged research in this area with other Lepidoptera (Karlson and Butenandt, 1959). Sex attractants recently characterized and identified include those of the cabbage looper, Trichoplusia ni (Hübner), (Berger, 1966) and the pink bollworm (Jones, Jacobson, and Martin, 1966).

Sex attractants have been used successfully in surveying field populations (Holbrook, Beroza, and Burgess, 1960) and the possibilities of using sex attractants for control of insect pests have been discussed by Götz (1951), Beroza and Jacobson (1963), and Knipling and McGuire (1966). Field evaluations of Lepidoptera sex attractants, used alone

or in combination with black light, have given encouraging results (Berger et al., 1964; Henneberry and Howland, 1966) and the possibility of using sex attractants in combination with chemosterilants in the field is currently of great interest (Knipling and McGuire, 1966).

Chemosterilization of Insects

The eradication of the screw-worm fly, Cochliomyia hominivorax (Coquerel), from the southeastern United States through the release of males sterilized by gamma radiation (Knipling, 1960) stimulated a search for chemicals capable of inducing sterility in insects (Lindquist, 1961). LaBrecque, Adcock, and Smith (1960) demonstrated the ability of several chemical compounds to cause sterility in house flies, and these chemicals were termed chemosterilants (LaBrecque, 1961). Chemosterilants act on insects in three principal ways: (i) by causing failure to produce ova or sperm; (ii) by causing death of sperm and ova after they have already been produced; (iii) by inducing dominant lethal mutations, or severely injuring the genetic material in the sperm and ova (Smith et al., 1964). The last-named mode of action, characteristic of the radiomimetic compounds, is of special interest because sperm and ova remain alive but zygotes formed do not complete development. Males sterilized in this way compete effectively with normal males for available females, and are able to satisfy the mating requirements of these females by the transfer of motile sperm (Smith et al., 1964). LaBrecque (1965) reported that 112

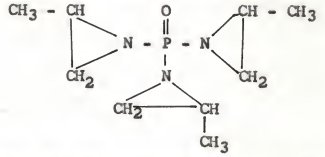
chemicals had the ability to sterilize insects and this number continues to grow (Kohls, Lemin, and O'Connell, 1966). Antimetabolites and alkylating agents have shown the greatest promise to date as insect chemosterilants (Smith et al., 1964). Antimetabolites are compounds which have the ability to substitute for, but not satisfactorily replace, compounds essential to metabolism and cell development (Woolley, 1952). Antimetabolites have been effective in inducing sterility only in female insects (Mitlin, Butt, and Shortino, 1957; LaBrecque et al., 1960; Crystal, 1963; Kilgore and Painter, 1966), and are considered to be of less potential value as chemosterilants than the alkylating agents.

Alkylating agents replace hydrogen on an organic molecule with an alkyl group (Ross, 1962). When this replacement takes place within fundamental genetic material the effect is similar to that produced by irradiation (Alexander, 1960). Wheeler (1962), Timmis (1961), and Kilgore (1965) have discussed the biochemistry and mode of action of alkylating agents.

The alkylating agents which have been evaluated most thoroughly as insect chemosterilants are apholate (2,2,4,4,6,6-hexakis(1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2,4,6-triazatriphosphorine), tepa (tris(1-aziridinyl)phosphine oxide), thiotepa (tris(1-aziridinyl)phosphine sulfide), and metepa (tris[1-(2-methyl) aziridinyl]phosphine oxide), the latter being considered the least hazardous to handle.

Metepa is a highly reactive tri-functional deriv-

ative of phosphorous oxychloride and propylene imine. The reactive functions of metepa are the three-membered imine rings shown in the structural formula below (Anonymous, 1962). The chemical in technical form is comprised of 92 per cent reactive imine, is a straw-colored liquid with a boiling point between 118 C and 125 C, and has a specific gravity of 1.079. Metepa is completely soluble in water and all common organic solvents (Anonymous, 1962). Like all aziridine compounds, metepa rapidly loses reactivity in even mildly acidic solutions (Beroza and Borkovec, 1964).



Metepa has been used successfully to sterilize the stable fly (Harris, 1962), house fly (LaBrecque, Meifert, and Gouck, 1963), screw-worm (Gouck et al., 1963), gypsy moth (Collier and Downey, 1965), and the pink bollworm (Ouye, Garcia, and Martin, 1965).

I have been unable to find any references in the literature dealing with the effects of chemosterilants on the potato tuberworm, or on the use of the sterility method of control against this species in the field. Elbadry (1963) presented data on the effect of gamma radiation on the tuberworm under laboratory conditions. He was able to sterilize emerging adults by exposing mature male and female

pupae to 15,000 and 18,000 r respectively, but sterilized males were not fully competitive with normal males. Elbadry (1964) suggested that if a program involving the use of radiation-sterilized tuberworms was ever undertaken females should be utilized rather than males.

The specialized facilities required, and the high initial cost involved in the use of radiation for inducing sterility in insects would make this approach impractical in Bermuda at present. Accordingly, the research reported here was restricted to an investigation of the potential value of chemically induced sterility as a method for controlling the potato tuberworm.

MATERIALS AND METHODS

The research reported here was conducted in the insectary and other facilities of the University of Florida, Gainesville, during the period January, 1966, to February, 1967.

Rearing Procedures

A supply of potato tuberworms obtained from the Potato Investigations Field Station at Hastings, Florida, was used to start a culture. Rearing was carried out at a temperature of 26.5 C ± 2.5° under continuous light of an intensity of approximately 15 ft-c. Rearing was most successful when the relative humidity was between 40 and 60 per cent, but in the absence of equipment for humidity control this range was not always achieved.

The methods developed by Finney et al. (1947) were used as the basis for the rearing program but many modifications were necessary in order to produce a continuous supply of healthy insects with a minimum of labor.

Small Irish potatoes (approximately 4 cm in diameter) were used throughout as the rearing medium. Tubers were punctured by rolling them over a board through which 15-gauge nails, spaced 1 cm apart, were projecting 1 cm.

After trials with several different cages, a cylindrical, bulk ice cream container (23.5 cm diameter; 9.5

liter capacity) was selected for use as a rearing cage (Fig. 1). A piece of waxed paper was placed on the bottom of the container, and a thin layer of sifted sand was spread over this. Two layers of potatoes, about 2.5 kg in all, were suspended on hardware cloth (1.5 mesh per cm) supported by steel rods (0.3 cm in diameter), pushed through the cardboard walls of the container. A top of unbleached muslin was held in place by the metal ring normally used with tops of containers of this type.

A modification of the ice cream container was also used in making egg recovery cages (Fig. 2). The cardboard cylinder was cut to a height of approximately 7.5 cm. A top and bottom were made by soldering wire screening (6 mesh per cm) to metal rings supplied with the containers. A piece of unbleached muslin was soaked in water then stretched tightly over the screen. A piece of waxed paper was placed on the cloth and held down by a wooden disc. The solid backing for the muslin encouraged moths to lay eggs on the cloth, and the waxed paper prevented the eggs from becoming stuck to the wooden backing disc. The egg cages were stocked with approximately 500 moths of mixed sex, and kept in total darkness, except during periods of handling. A feeder containing 10 per cent sucrose solution was provided.

New cultures were started by placing an egg sheet over each layer of potatoes in the rearing cage. The sheets were removed after 6 days when all the eggs had hatched. On completion of development, larvae pupated in the sand at the

Fig. 1.--Potato tuberworm rearing cage made from bulk ice cream container. Sand for pupation shown at bottom center, and muslin top at bottom left.

Fig. 2.--Egg recovery cage made from bulk ice cream container with wire screen top and bottom.

bottom of the cage (Fig. 3). They were removed from the rearing cage 18 to 22 days after the culture was started. This was easily accomplished by peeling away the mat of sand-covered cocoons from the waxed paper sheet on the bottom of the cage.

Pupae were separated from their cocoons in the manner prescribed by Bartlett and Martin (1945). The mat of sand, cocoons, and pupae, was placed in a 15-cm household sieve and soaked for approximately 1 minute in a 2.5 per cent sodium hypochlorite solution. This freed the pupae from their cocoons; the sand fell through the sieve leaving the pupae behind. Pupae were then rinsed, first for 20 to 30 seconds in sodium thiosulphate to stop the action of the hypochlorite, then in water for 1 minute, and finally in 60 per cent isopropyl alcohol to expedite drying. Between 2,000 and 2,500 pupae were recovered from each cage.

A proportion of the pupae obtained were placed individually in 10-dram shell vials closed with cork stoppers. Moths emerging in these vials were grouped according to sex and age, and held under continuous light until required for experimentation. It was much easier to sex moths than pupae, and by working with individual containers the virginity of the moths was assured.

Pupae not required for experimental purposes were used to provide moths for the egg cages. These pupae were placed in a shallow container made from the bottom one third of a paper ice cream tub (0.47 liter). An umbrella-like hood

Fig. 3.--Tray of potato tuberworm pupae in sand spread over waxed paper. Approximately 2,500 pupae were produced per tray.

was suspended over the pupae (Fig. 4). The entire container was placed in a wide-mouth jar (1.9 liter), with a wire screen top. When adults emerged they settled on the sides and top of the jar. They were removed daily after being anesthetized with CO_2. The hood prevented anesthetized adults from falling back among the pupae.

This rearing procedure proved very efficient, with more than 100,000 moths being produced during the course of the project. By using the disposable containers for rearing cages, it was possible to control the bacterial disease caused by Serratia marcescens Bizio--a problem which initially threatened to prevent the production of healthy tuberworms.

Morphology of the Reproductive System

Two-day-old moths were used in morphological studies. Wings of the moths were clipped off and scales on the body removed by gently rolling moths on sticky cellophane tape (Tedders and Calcote, 1967). Dissections were made in water, using a stereoscopic microscope. Measurements were taken from freshly dissected specimens.

Ratio of Sexes

The ratio of sexes was determined for 15 samples of pupae selected at random from among the 58 rearing cages used during the course of this study. In addition, 20 small cultures of tuberworm were set up in mason jars (0.47 liter), each with a single potato tuber stocked with 50 tuberworm eggs, and the resulting pupae sexed.

Fig. 4.--Container used to recover newly emerged potato tuberworm moths.

Reproductive Behavior

Parthenogenetic reproduction

Two-hundred virgin females (four groups of 50, each group from a different generation) were caged (unfed) in 12-dram snap-cap plastic vials with a disc of black cotton cloth inset in the cap (Fig. 5). The moths were kept in continuous darkness for the duration of their lives. Records were taken on fecundity and fertility.

Criterion for successful mating

Throughout this study, the presence of a spermatophore in the bursa copulatrix of a female moth, determined by dissection under a stereoscopic microscope, was used as a criterion for successful mating. More than 2,000 dissections were made for this purpose.

Number of spermatophores transferred during single mating

Two-day-old virgin male and female moths were caged as individual pairs in 12-dram vials and held in total darkness. Moths were observed continuously, using a heavily hooded flashlight. Records were kept on the time of start and finish of copulation. Mated pairs were killed immediately on termination of copulation and a count made of spermatophores passed. Two replicates were run, each with 75 pairs.

Influence of age on mating

In order to determine the earliest age at which tuberworm moths would mate, adults of the sex being tested were caged within 15 minutes of eclosion with two 2-day-old

Fig. 5.--Containers used to cage potato tuberworm moths during behavior studies. Left, 0.21-liter food cup. Right, 12-dram vial with cloth inset used in lid.

virgins of the opposite sex in 12-dram vials and held in continuous darkness. A 10 per cent sucrose solution was provided. The percentage of insects that mated successfully within 3, 6, 9, 12, and 24 hours was determined. Forty moths of each sex were used for each time interval.

Lifetime successful matings

Tests were run to determine the maximum number of times members of both sexes would mate successfully in the course of a lifetime when provided with (i) a single mate (determined for fed and unfed moths), and (ii) a continuous fresh supply of virgin partners.

Single pair studies were carried out in 12-dram vials with four replicates being run, each one comprised of 10 pairs. In tests with multiple mates, 1-day-old virgin moths of the sex being evaluated were caged in 0.21-liter food cups (Fig. 5) with three 2-day old virgins of the opposite sex. A 10 per cent sucrose solution was provided. Every 2 days two or three fresh virgins were added to the cage, until the death of the test insect. Fifty moths of each sex were evaluated in this way.

An additional measure of potential successful lifetime matings of male tuberworm moths was obtained by caging a single 1-day-old virgin male with two 2-day-old virgin females in a 12-dram vial. The females were replaced daily with two fresh virgins for the lifetime of the male. Twenty males were tested in this way.

All of these mating studies were run in continuous

darkness, except for a brief daily period during handling.

Influence of photoperiod on mating and fecundity

The influence of four light regimes on mating and fecundity of the potato tuberworm was investigated. The regimes were (i) continuous light, (ii) continuous dark, (iii) 12 hours' light (6:00 AM to 6:00 PM) and 12 hours' dark, and (iv) alternating periods of 1 hour light and 1 hour dark.

All tests were carried out in a Parce bioclimatic chamber, with the temperature maintained at 26.6 C $\pm$ 0.5° and relative humidity at 75% $\pm$ 5%. Continuous light was maintained in the chamber and three small portable chambers, set up within the large unit, were used to provide the remaining three light regimes. Air from the bioclimatic chamber was continuously drawn through these small chambers to assure constant conditions for all treatments.

One-day old virgins, held in continuous light during pupation and eclosion, were caged as individual pairs in 12-dram snap-cap plastic vials. A black cotton disc was inset in the cap to serve as an oviposition site (Fig. 5). Food was not provided. Vials were inverted to allow incident light from fluorescent (daylight) tubes to pass directly into them producing an intensity of 120 ft-c within the vials.

Ten vials comprised one replicate with each treatment replicated four times. Tests were run for 7 days when mating was determined. Fecundity was determined by a direct

count of all eggs in each vial. These counts were also made at the end of 7 days since investigations by Labeyrie (1957a), and my own findings, had shown that unfed potato tuberworm moths completed egg laying within 4 days of mating.

Fecundity studies were also run using 10 pairs of moths per cage (0.47-liter mason jar) with the light regimes being (i) continuous light, and (ii) continuous dark. Three replicates were run under each regime. Mating was not determined in these tests.

An additional experiment was conducted to determine whether continuous light would delay mating. Two-day-old males and females were caged as individual pairs in 12-dram vials and held under continuous light for 24 hours, after which mating was determined. A similar procedure was followed with moths held under continuous dark. Four replicates, each of 10 pairs, were used for each light regime.

Extraction and Bioassay of Sex Attractant

Extraction

Abdomens of virgin female potato tuberworm moths were homogenized with a mortar and pestle using a small amount of methylene chloride to facilitate grinding. The homogenate was filtered through Whatman no. 1 qualitative filter paper into a 50-ml beaker and the residue re-extracted and filtered. The excess methylene chloride was evaporated by placing the beaker in a water bath at 45 C and directing a gentle stream of air on the filtrate. The oily residue was diluted with methylene chloride to a convenient volume and

the solution held at 7 C until required. Two-day-old virgins were used for most extractions, but moths of different ages were used in some tests for comparative purposes.

Bioassay

The bioassay procedure followed was basically the same as that developed by Shorey, Gaston, and Fukuto (1964) for use with the cabbage looper.

Two-day-old unfed virgin males, held under continuous light from eclosion, were used in all assays. Five males were placed in a clear plastic cylinder 13 cm high and 5 cm in diameter, closed at the top and bottom by plastic screen (6 mesh per cm) (Fig. 6).

Bioassays were run initially on a stock methylene chloride extract of 100 2-day-old virgin females. A graded series of concentrations of the stock solution was prepared, with concentrations being expressed in terms of female equivalents. A known number of female equivalents was pipetted onto a 4.5-cm diameter filter paper disc and the disc placed on top of a clean disc of the same size in a Büchner funnel (4.8 cm inside diameter). A stream of air from an outside source was delivered through the funnel at 800 ml per minute. A bioassay cage containing five males was placed on the lip of the Büchner funnel (Fig. 6) and the response of the males recorded at 15 and 30 seconds. Males vibrating their wings or trying to copulate with other males in the cage were considered to have responded positively to the attractant. The higher of the two readings was used when data were compiled.

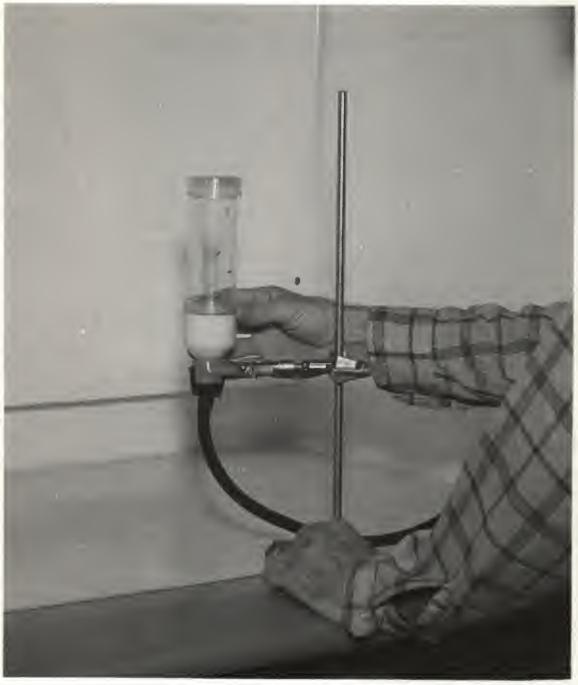


Fig. 6.--Bioassay apparatus. Male moths are caged in clear plastic cylinder which rests on the lip of the Buchner funnel. Sex pheromone is placed on filter paper disc in the funnel. Air is provided from outside source.

All assays were carried out under artificial light of approximately 3 ft-c intensity. Most of the tests were run at night between 7:00 PM and 1:00 AM, but certain series of assays were run at 12-hour intervals. Cages not in use were held under an exhaust hood in a room apart from the one in which assays were run. No cage was used more frequently than once every 20 minutes, and in no case more than three times in one night.

Trapping males with sex attractant

Traps were made from two paper cups (0.71 liter), complete with plastic lids. The bottoms were cut out of the cups so that the narrow end of one could be telescoped over the narrow end of the other. A hole 3.2 cm in diameter was cut in each lid and a hole, large enough to accept a no. 10 cork, bored in the side of the trap. Tanglefoot® was spread evenly over the entire inside of the trap. A 7.5-cm filter paper disc was folded to provide a fluted surface and fastened to the small end of a no. 10 cork. Twenty female equivalents, carried in 2 ml of methylene chloride, were pipetted onto the filter paper which was then introduced through the hole in the side of the trap (Fig. 7).

Trapping tests were run in a cage 1.2 m long, 1.2 m wide, and 1.8 m high. The temperature in the room holding the cage was 26.5 C ± 1° and the relative humidity 60% ± 5%. A cord was stretched across the center of the cage 1 m above the floor and a baited trap and a control (2 ml of methylene chloride) suspended from this. The traps were spaced 0.6 m

Fig. 7.--Trap used with crude extract of sex pheromone from female potato tuberworm moth. Pheromone extract is placed on filter paper, which is then inserted in trap. Inside of trap coated with Tanglefoot.

apart and were aligned in an identical manner with the openings horizontal with the floor. A small fan set up outside the cage provided a gentle flow of air through the traps.

Fifty 2-day-old, unfed, virgin male moths, kept since eclosion in continuous light, were used in trapping tests. Moths were introduced into the cage at the start of a 1-hour light (6 ft-c) period. Twelve hours of darkness followed by 11 hours of light completed the light regime. Catches were recorded at the time of change from one light condition to another. Moths not trapped were removed from the cage before the start of each replicate. Four replicates were run, each one with a new trap and a fresh aliquot of attractant.

Trapping was also attempted in a room approximately 4.6 m x 3.4 m x 2.4 m (high), maintained at a temperature of 26.5 C ± 2.5° and a relative humidity of 60% ± 10%. Natural light conditions existed (approximately 12 hours' light, 12 hours' dark). One trap baited with 50 female equivalents and one control trap were suspended 1.2 m from the floor; air movement was provided by a small fan. Traps were set at 6:00 PM and removed 24 hours later. An estimated 500 moths of mixed sex and age were in the room at the start of each of four replicates.

Chemosterilant Experiments

Technical metepa (92 per cent) dissolved in reagent-grade acetone was used in all experiments and was supplied in required concentrations by the Laboratory of Insects

Affecting Man and Animals, Entomology Research Division, USDA, Gainesville, Florida. Metepa was applied topically on the thoracic sternum of 1-day-old virgin moths, previously anesthetized lightly with ether. Application was made with a repeating micro-syringe delivering 1 μl. Dosages were expressed in micrograms of technical metepa per moth.

Fecundity and longevity were determined by caging moths as individual pairs in 12-dram snap-cap vials with a cloth inset serving as an oviposition site (Fig. 5). Mating was determined by spermatophore count.

In preliminary tests, treated moths were paired with an untreated moth of the opposite sex immediately after application of the chemosterilant. Subsequently, treated moths were held 24 hours before pairing. Egg counts, hatch, and mating were determined 8 days after pairing of moths--the exception being when females died prior to this time, in which case spermatophore counts were made on the day of death. Tabulated data are based upon four replicates, each consisting of 10 treated moths. Treated moths were held in continuous darkness, except for brief periods of handling, at a temperature of 26.5 C $\pm$ 2.5°. Humidity was not controlled.

Statistics

Chi-squares, "t" tests, "F" tests, and standard errors were calculated according to the methods of Snedecor (1956). In chemosterilant studies lethal dose (LD) and effective dose (ED) are expressed in micrograms per moth

(except for a single case in which mortality of males and females was compared on a dose per unit weight basis) and the safety factor (SF) for metepa was determined by the method of Chang and Borkovec (1966).

RESULTS AND DISCUSSION

Morphology of the Internal Reproductive System

Male

The male reproductive system of P. operculella (Fig. 8) consists of paired testes enclosed in a common scrotum, paired vas deferens, enlarged along their course to form the seminal vesicles, a ductus ejaculatorius duplex uniting caudaly with the ductus ejaculatorius simplex and terminating with the endophallus within the aedeagus. Paired accessory glands extend from the cephalad end of the ductus ejaculatorius duplex.

Testes.--The testes of P. operculella (Fig. 8A) are so closely united that they appear as a single round organ lying dorsally in the abdomen at about the fourth segment. They are easily recognized by their size (0.7 mm to 0.8 mm) and their dull purple color. The form of the testes of the tuberworm conforms to Cholodkovsky's (1884) class D group testes, typified by Pieris. The dark testes are readily visible through the dorsal side of the abdominal wall of fourth instar male larvae, and in pupae.

Vas deferens and seminal vesicles.--Paired vas deferens (Fig. 8B) leave the base of the testes in close proximity. The ducts are 0.15 mm to 0.2 mm in diameter at the point of junction with the testes, but narrow as they

Explanation of Fig. 8

A. Paired testes, fused in common scrotum

B. Vas deferens

C. Seminal vesicle

D. Ductus ejaculatorius duplex

E. Accessory glands

F. Second secretory region of primary simplex

G. First secretory area of primary simplex

H. Cuticular simplex

I. Aedeagus

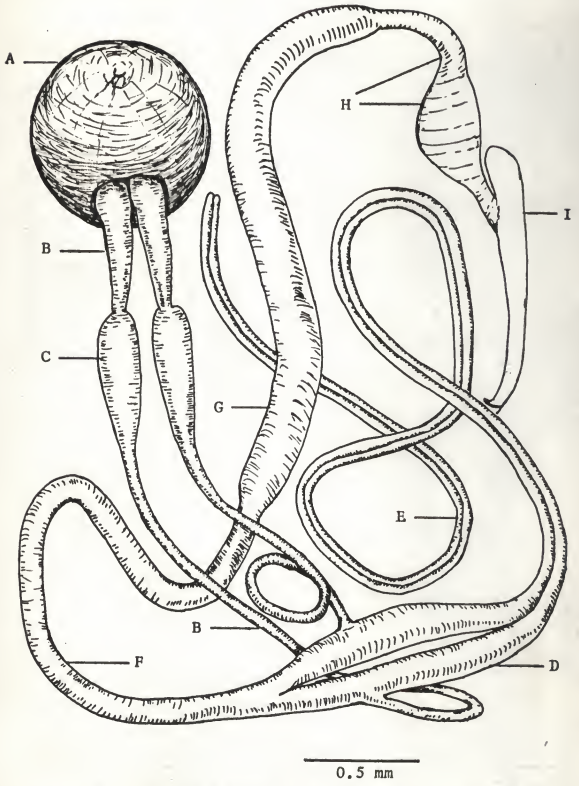


Fig. 8.--Reproductive system of male potato tuberworm moth.

enter the dilated seminal vesicles (Fig. 8C). The lower portions of the vas deferens, emerging from the caudal end of the seminal vesicles, are only 0.06 mm to 0.08 mm in diameter. Callahan and Chapin (1960) report that in the Noctuidae the vas deferens are capable of extreme distension along their entire length, when sperm bundles pass along the ducts. This condition was not observed in the tuberworm, but in the absence of serial morphology studies the possibility of such an occurrence cannot be excluded.

The seminal vesicles (Fig. 8C) are thin-walled ducts in which sperm are stored (Norris, 1932). In P. operculella these ducts are approximately 0.6 mm long and 0.15 mm to 0.2 mm in diameter at their widest point.

The total length of the vas deferens, including the incorporated seminal vesicles is about 3.0 mm.

Ductus ejaculatorius duplex.--The caudal ends of the vas deferens enter the large ductus ejaculatorius duplex (Fig. 8D) at a point approximately midway along the branches of this gland. The individual branches of the ductus ejaculatorius duplex are about 0.15 mm in diameter and 1.2 mm long. There is often a distinct separation between the two branches for at least a portion of their length.

Callahan and Chapin (1960) found the ductus ejaculatorius duplex to be filled with a mixture of sperm and accessory gland fluid in noctuid moths, and Norris (1932) considered this gland to be the principal sperm reservoir in the Phycitidae. In the tuberworm the gland was distended with

sperm and a fluid, presumed to be the product of the accessory glands.

Accessory glands.--The cephalad ends of the branches of the ductus ejaculatorius duplex continue as the accessory glands (Fig. 8E). These glands are united along their entire length and wind through the abdominal cavity, ending blindly in the anterior portion of the abdomen. The accessory glands are 7 mm to 9 mm long and 0.06 mm to 0.08 mm wide. They are by far the longest organ in the male reproductive system.

The functions of the secretions of the accessory glands are not completely understood. It is known that fluid from these glands is incorporated into the spermatophore and it has been suggested that the secretion might be a nutritive medium for sperm (Norris, 1932), or might contain activating substances capable of initiating rhythmic movements of the female reproductive ducts,and thereby assist in sperm transmission (Callahan and Chapin, 1960; Davey, 1958).

Ductus ejaculatorius simplex.--A single, long duct, the ductus ejaculatorius simplex extends from the caudal end of the ductus ejaculatorius duplex to the aedeagus. Callahan (1958), and Callahan and Chapin (1960) recognized two distinct morphological regions in this duct in the Noctuidae, a cephalad primary region, of secretory function, and a caudad cuticular segment, where precursors of the spermatophore are moulded. Callahan and Cascio (1963) later subdivided the primary secretory segment into first and second secretory

areas. In much earlier works Norris (1932), working with Anagasta kuehniella (Zeller) described four different secretory regions in this duct, and Musgrave (1937), also working with A. kuehniella, differentiated eight regions--all determined histologically.

In the potato tuberworm four regions can be recognized in the ductus ejaculatorius duplex on the basis of gross morphology. These regions have been described in the terminology of Callahan and Cascio (1963). The first region, the secondary secretory area of the primary simplex, extends approximately 4 mm from the ductus ejaculatorius and is 0.1 mm to 0.12 mm in diameter (Fig. 8F). It includes the first three unpaired glands of Norris (1932).

The second region (Fig. 8G), the first secretory area of the primary simplex, is approximately 0.2 mm in diameter and 3 mm long. It is normally filled with a viscous, yellow fluid. This would appear to be the fourth, or lower unpaired gland of Norris (1932).

The third region of the duct is thick-walled, short (0.5 mm to 0.8 mm), and narrow (0.1 mm to 0.2 mm in diameter), and together with the inflated fourth region forms the cuticular simplex (Fig. 8H).

The ductus ejaculatorius simplex in the tuberworm is far more simple, from a morphological standpoint, than in the Noctuidae, or even in the Phycitidae. There is nothing comparable to the area where the frenum of the spermatophore is found in the Noctuidae (Callahan and Chapin, 1960), or to

the "hollow horns" in Plodia and Anagasta utilized in moulding the ornate spermatophores of those species. It is probable, however, that the third and fourth regions of the ductus ejaculatorius simplex in the tuberworm are involved in the moulding of the resilient spermatophore from materials elaborated by the secretory region of this same duct.

Aedeagus.--The aedeagus (Fig. 8I) is approximately 1.2 mm long and 0.12 mm deep at the cephalad end, tapers posteriorly, and is slightly curved. It is sclerotized only along its dorsal and lateral surfaces, the ventral surface being membranous. Two sclerotized structures in the form of small recurved claws occur at the terminus of the aedeagus, with an opening between them. A thin, lightly sclerotized plate occurs dorsally at the terminus of the aedeagus and projects over the opening of this organ. The endophallus terminating the ductus ejaculatorius duplex occurs within the aedeagus and is everted during copulation.

Female

The female reproductive system of P. operculella (Fig. 9) consists of the paired ovaries opening into lateral oviducts, which unite to form the common oviduct terminating with the ovipositor. A spermatheca and paired accessory glands open dorsally on the common oviduct. A prominent bursa copulatrix has an exterior opening independent of the common oviduct, the only junction between these two organs being provided by the ductus seminalis.

Ovaries.--The abdominal cavity is dominated by the

Explanation of Fig. 9

A. Ovariole

B. Calyx

C. Lateral oviduct

D. Common oviduct

E. Ovipositor

F. Posterior apophyses

G. Spermatheca

H. Spermathecal duct

I. Spermathecal gland

J. Accessory glands

K. Accessory gland reservoir

L. Corpus bursae

M. Ductus bursae

N. Ostium bursae

P. Anterior apophyses

R. Ductus seminalis

S. Bulla seminalis

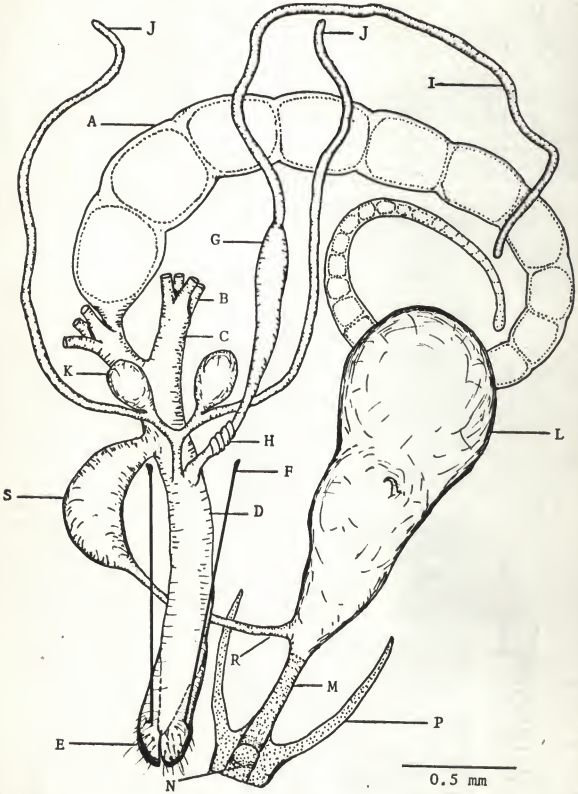


Fig. 9.--Reproductive system of female potato tuberworm moth.

paired ovaries, each with four polytrophic ovarioles (Fig. 9A). The ovarioles are 4 mm to 5 mm long and are coiled within the abdominal cavity. Anteriorly they fuse to form a common terminal filament, but there is no suspensory ligament.

An average of eight fully developed eggs was present in each ovariole and an additional 24 to 30 developing eggs were discernible. Labeyrie (1957a) reported that tuberworm moths produced an average of 223 eggs when fed, and this would indicate that virtually all of the potential eggs of this species are visible at the time of adult eclosion. Eidmann (1931) placed the Lepidoptera into the following three groups on the basis of the number of ripe eggs in the ovaries at eclosion: (i) those species with very few ripe eggs at emergence, typified by butterflies and moths with a long adult life; (ii) those that had a two-or three-fold increase in ripe eggs in the imago, as is the case with most Heterocera; and (iii) those with all eggs fully developed at eclosion, as occurs in some members of the families Bombycidae and Lymantriidae. The tuberworm can best be placed in the second group.

Oviducts.--The four ovarioles of each ovarium meet caudaly at the calyx (Fig. 9B) and enter a short (0.2 mm to 0.3 mm) lateral oviduct (Fig. 9C). The lateral oviducts unite to form the common oviduct (Fig. 9D) which terminates with the ovipositor (Fig. 9E)--a fusion of the ninth and tenth abdominal segments everted by the posterior apophyses (Fig. 9F).

Spermatheca.--The spermatheca (Fig. 9G) is an elongated, thin-walled chamber joined to the dorsal side of the common oviduct by a tightly spiraled duct (Fig. 9H). The spermatheca proper is approximately 1.0 mm long and 0.12 mm in diameter at its broadest point. A long narrow spermathecal gland (Fig. 9I) extends 3.0 mm to 4.0 mm from the cephalad end of this organ.

Weidner (1934) has shown that copulation triggers the activity of the spermathecal gland in Bombyx mori resulting in the flow of secretion into the spermathecal duct and Davey (1965) suggests that this fluid may serve to orient sperm. Norris (1932) proposed that sperm were nourished by the secretion of the spermathecal gland.

Accessory glands.--A pair of accessory glands, attaining a length of 2.0 mm to 3.0 mm, wind among the reproductive organs in the abdominal cavity (Fig. 9J). At the base of each gland there is a thin-walled reservoir (Fig. 9K) capable of great expansion. Below the reservoir the accessory glands unite and enter on the dorsal side of the common oviduct, proximal to the spermathecal duct.

The secretion of the accessory glands is used in cementing eggs to the oviposition site (Davey, 1965).

Bursa copulatrix.--The bursa copulatrix (Fig. 9 and 10) is very prominent in P. operculella. It is comprised of the corpus bursae (Fig. 9L), ductus bursae (Fig. 9M), and the ostium bursae (Fig. 9N).

The corpus bursae is a heavy-walled, transparent

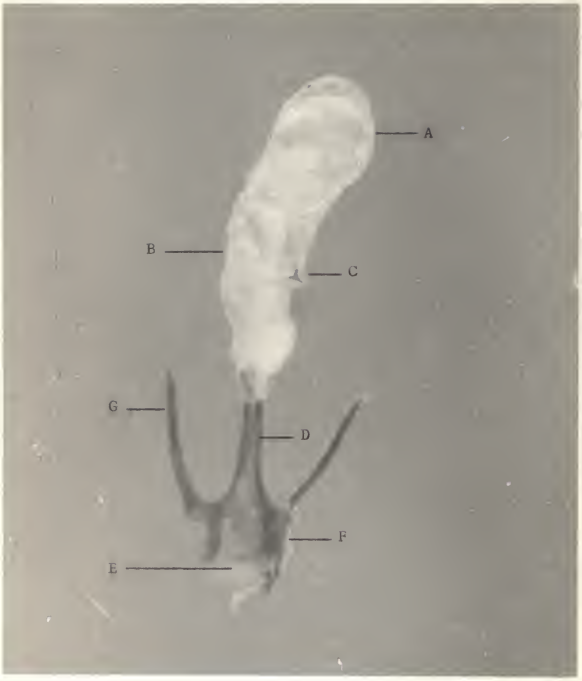


Fig. 10.--Photomicrograph of bursa copulatrix of potato tuberworm moth. A, corpus bursae; B, region of spermatophore; C, signum; D, ductus bursae; E, ostium bursae; F, genital plate; G, anterior apophyses. Enlarged approximately 60 times.

pouch and in fertilized females is 1.5 mm to 1.7 mm long and 0.6 mm to 0.8 mm in diameter at its broadest point. In newly emerged virgins it is flattened, but becomes partially distended in older virgins (Fig. 11) as the result of being filled with a viscous, yellow fluid of undetermined origin. A single chitinized, decurved tooth, the signum (Figs. 10 and 12) occurs on the inner ventral surface of the corpus bursae and projects into the lumen. This tooth serves to hold the spermatophore, and possibly to provide pressure to assist in the evacuation of sperm.

The chitinized ductus bursae, which receives the aedeagus during copulation, is 0.08 mm to 0.1 mm in diameter and 0.4 mm to 0.5 mm long. It is expanded caudaly into a sclerite which Poos and Peters (1927) termed the genital plate. This plate lies ventrally in the abdomen just anterior to the eighth segment. Two long projections, the anterior apophyses, arise from the anterior edge of the plate (Fig. 9P), and two smaller projections (not shown in Fig. 9) from the posterior edge, the latter two being fused with the eighth abdominal segment. Muscles attached to the anterior apophyses stabilize the ductus bursae and also evert the eighth abdominal segment during oviposition. The external opening of the bursa copulatrix, the ostium bursae, occurs at the posterior edge of the genital plate and opens into the cuticular fold between the seventh and eighth abdominal segments.

Ductus seminalis.--A narrow duct (0.04 mm to 0.06

Fig. 11.--Photomicrograph of bursa copulatrix of (left) mated, and (center) unmated moths, and two spermatophores. Bursa copulatrix from unmated moth is not fully distended. Enlarged approximately 45 times.

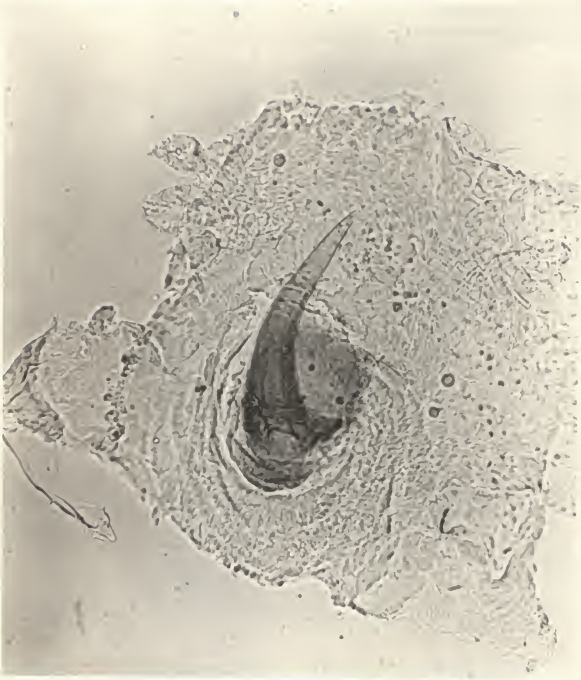


Fig. 12.--Chitinized signum of bursa copulatrix of potato tuberworm moth. Enlarged approximately 900 times.

mm), ductus seminalis (Fig. 9R) leaves the corpus bursae at a point just above the cephalad end of the ductus bursae. The duct dilates to form the bulla seminalis (Fig. 9S) before entering the dorsal side of the common oviduct at a point just anterior to the accessory gland duct. The ductus seminalis provides the pathway by which sperm reaches the spermatheca.

In some Lepidoptera the bulla seminalis is a distinct organ set off from the ductus seminalis (Tedder and Calcote, 1967). The function of the bulla seminalis is not known (Wellso and Atkinson, 1962).

The spermatophore

The chitinous spermatophore is 0.7 mm to 0.9 mm long, 0.35 mm to 0.45 mm wide at the broadest point (Fig. 11), and roughly pear-shaped. The extended neck, typical of the spermatophores of many Lepidoptera, is reduced to a very small nipple-like structure through which the sperm escapes. The walls are transparent and resilient, and the sperm and accessory gland fluids appear as an opaque core within the spermatophore.

The spermatophore is placed within the corpus bursae of the female in such a way that the sperm exit is in direct contact with the ductus seminalis. Williams (1941) placed Lepidoptera into three groups on the basis of the position of the spermatophore within the corpus bursae. The arrangement which exists in the tuberworm is typical of Williams' class A Lepidoptera.

The spermatophore is held against the inner dorsal surface of the corpus bursae by the prominent signum. The sharp point of the signum presses against the ventral surface of the spermatophore at a point approximately two thirds along its length causing a depression at the point of contact. The expanded blunt end of the spermatophore extends beyond the signum. Stitz (1901) suggested that sperm escaped through punctures made by the signa, but in the hundreds of spermatophores examined during my study no evidence was seen of this.

In cases where multiple matings occur, the initial spermatophore, empty and flattened, is forced forward, and the newly introduced spermatophore is positioned in the neck of the corpus bursae. As many as six spermatophores were found in a single ductus bursae, five collapsed and flattened, and more or less nested within one another, and the most recently deposited one occupying the neck of the corpus bursae.

Williams (1938) believed spermatophores could be dissolved by enzymes within the ductus bursae, but no indication of this was found in the potato tuberworm.

Ratio of Sexes

Tables 1 and 2 record the ratio of sex in potato tuberworm pupae obtained from (i) samples selected at random from mass rearing cages, and (ii) small rearing cages holding one tuber stocked with 50 eggs.

There was a significant departure from the expected

1:1 sex ratio in 13 of 15 samples collected from mass rearing cages (Table 1), with males predominating in every case. This trend was reinforced by the extremely high Chi-square value for the pooled data. The fact that the 1:1 ratio was upheld in pupae reared in small cages (Table 2), under less competitive and crowded conditions, suggests the possibility of some pressure acting differentially upon females in the egg or larval stage under mass rearing conditions.

Table 1.--Ratio of sexes of potato tuberworm pupae in 15 samples selected randomly from among 58 mass rearing cages, with number expected under a 1:1 hypothesis and value of Chi-square.

Sample size	Males	Females	Expected no.	Chi-square
897	522	375	448.5	24.1**[a]
291	182	109	145.5	18.3**
871	498	373	435.5	17.9**
597	336	261	298.5	9.4**
896	478	418	448.0	4.0*[b]
389	224	165	194.5	8.9**
504	281	223	252.0	6.7*
376	212	164	188.0	6.1*
311	175	136	155.5	4.9*
425	235	190	212.5	4.8*
539	291	248	269.5	3.4
408	228	180	204.0	5.6*
389	234	155	194.5	16.0**
457	250	207	228.5	4.0*
267	144	123	133.5	1.7
Pooled 7,617	4,290	3,327	3,808.5	121.7**

[a]exceeds the 0.01 point.
[b]exceeds the 0.05 point.

The imbalance in sex ratios was not investigated, but a possible explanation for this condition might be found in the effect of the bacterium Serratia marcescens on the tuberworm. This organism has always been a troublesome pathogen in tuberworm mass cultures (Finney et al., 1947), and

Table 2.--Sex ratio of all of the potato tuberworm pupae obtained from 20 small rearing cages, each stocked with 50 eggs, along with the number expected under the 1:1 hypothesis and values of Chi-square.

Sample size	Males	Females	Expected no.	Chi-square
41	23	18	20.5	0.61
39	18	21	19.5	0.23
35	14	21	17.5	1.40
40	18	22	20.0	0.40
37	19	18	18.5	0.03
41	19	22	20.5	0.22
32	19	13	16.0	1.13
33	15	18	16.5	0.27
37	13	24	18.5	3.27
42	20	22	21.0	0.09
40	17	23	20.0	0.90
43	24	19	21.5	0.58
44	23	21	22.0	0.09
32	15	17	16.0	0.13
37	18	19	18.5	0.24
38	17	21	19.0	0.42
44	20	24	22.0	0.36
31	18	13	15.5	0.29
35	14	21	17.5	1.40
45	25	20	22.5	0.56
Pooled 766	369	397	383.0	1.02

was a severe problem at the start of this program. The bacterium was brought under a high level of control and visible losses were negligible. Mortality among first instar larvae is very difficult to detect, however, and it is possible that mortality was occurring at this stage throughout the rearing program, with females being affected more than males.

Microsporidian diseases, to which the tuberworm is subject (Steinhaus and Hughes, 1949), have been known to influence sex ratios in insects in favor of males (Labeyrie, 1957b), but no trace of these organisms was found in the tuberworm cultures used in this program.

Wigglesworth (1955) has pointed out the importance of

environment on sex determination in Lepidoptera, citing the case of Talaeporia where high temperatures (30 C to 37 C) favored the production of males, while low temperatures (3 C to 8.5 C) favored females. The influence is explained by the effect of temperature on the movement of the X-chromosome during oogenesis. Female Talaeporia (as in all Lepidoptera) are heterogametic with the X-chromosome carrying the genes for maleness (Davey, 1965). High temperatures favor the appearance of the X-chromosome in the female pronucleus and lower temperatures cause it to appear in the polar body.

An effect of temperature on sex determination in the potato tuberworm (reared at 26.5 C $\pm$ 2.5°) similar to that of Talaeporia is a possibility, but would still leave unexplained the return to the 1:1 ratio in small cultures.

Reproductive Behavior

Parthenogenetic reproduction

Three virgin females out of a total of 200 caged produced viable eggs. One laid 37 eggs, with one hatching, a second laid eight eggs, with six hatching, and the third laid eight eggs with two hatching, giving a viability figure of 17 per cent. No attempt was made to rear the larvae and determine sex, since Picard (1913), Poos and Peters (1927), and Hofmaster (1949) had previously shown that offspring of both sexes could be expected from unfertilized eggs of this species.

Picard (1913) reported parthenogenetic reproduction

in approximately 9 per cent of the potato tuberworm females he tested (N = 100+), Poos and Peters (1927) 6 per cent (N = 161), and Hofmaster (1949) 7 per cent (N = 300). In every case the number of viable eggs produced per female was less than three.

The occurrence of parthenogenetic reproduction in this species is unlikely to have an important influence on the ability of the insect to survive in an area, or to seriously hinder an eradication program based upon the release of sterile males. The percentage of parthenogenetic females is too small, and the number of eggs laid by unmated females too low in comparison with mated females, as is shown in Table 3, to constitute a reliable substitute for sexual reproduction in the species.

Table 3.--Fecundity and fertility of mated and unmated tuberworm moths.

Condition	Sample size	Eggs		
		Mean ± SE	Range	% Hatch
Mated	185[a]	54.5 ± 2.1	0 - 124	95.4
Unmated	200	10.6 ± 0.9	0 - 73	0.4

[a]200 pairs were caged but 15 pairs did not mate.

Number of spermatophores transferred during a single mating

In order to use the presence of a spermatophore as a reliable measure of mating frequency, it was necessary to establish that only one spermatophore was passed at each successful mating. Table 4 records the results of an experiment used to test that premise, and to determine time spent in copulation.

Table 4.--Relationship between time in copulation and successful transfer of spermatophores during single mating of tuberworm moths. Two replicates, each of 75 pairs.[a]

Time in copulation in minutes	Number of females	
	Mated	With spermatophores
1 - 10	11	0
11 - 30	3	0
31 - 60	4	4
61 - 90	46	41
91 - 120	65	63
121 - 150	4	4
151 - 180	2	2
> 180	2	2

Mean for successful mating = 100.3 ± 3.5 minutes (N = 116)
Range for successful matings 43 - 424 minutes

[a]1 pair died in copulation and 12 pairs did not copulate.

In no instance was more than one spermatophore found in the bursa copulatrix of a female moth. This finding is consistent with that of Callahan (1958), Proverbs and Newton (1962), Dustan (1964), and Ouye, Garcia, Graham, and Martin (1965) for other Lepidoptera.

The minimum period in copula required for successful transfer of a spermatophore was 43 minutes, but some matings which lasted considerably longer than this failed to result in spermatophore transfer, as evidenced by data for the 61 to 90, and 91 to 120-minute pairing periods in Table 4. The mean time required for successful mating was 100.3 minutes. Dustan (1964) reported a mean time of 77 minutes for successful mating for 313 oriental fruit moths, Grapholitha molesta (Busck), and Ouye et al. (1964) 139 minutes for 28 matings in the pink bollworm.

Copulatory aberrations, in which mating pairs were not able to separate, were infrequent among tuberworm moths. Callahan and Chapin (1960) found evidence in noctuid moths indicating that the more complex the method of spermatophore transfer the greater the likelihood of copulatory aberrations. The low incidence of aberrant mating in P. operculella can probably be ascribed to the relatively simple mechanism of spermatophore transfer in this species.

Influence of age on mating

The results of this experiment are given in Table 5. No males or females mated during the first 3 hours after eclosion, but some individuals of both sexes were able to mate within 6 hours of eclosion. This precocious behavior is not unusual among Lepidoptera. The peach tree borer, Sanninoidea exitiosa (Say) (Smith, 1965), and silkworm moth (Makino, Satoh, and Inagami, 1956) will mate within an hour of eclosion. Ouye, Garcia, Graham, and Martin (1965) have reported that many pink bollworm moths mate on the night of emergence.

Five per cent of the males evaluated at 12 hours after eclosion were found to have mated twice within this period, and 10 per cent mated twice within 24 hours of eclosion.

Potato tuberworms must be sexed in the pupal stage to insure the virginity of test insects. Alternatively, pupae must be held in individual containers as was done in this study.

Table 5.--Elapse of time between adult eclosion and successful mating of potato tuberworm moths. Adult of sex being tested caged with two 2-day-old virgins of opposite sex. Run in continuous darkness. N = 40 for each sex of each age grouping. Ten per cent sucrose solution provided.

Age at dissection in hours	% Mating successfully	
	Males	females
3	0	0
6	7.5	5.0
9	52.5	45.0
12	75.0[a]	85.0
24	97.5[b]	100.0

[a]5% mated twice within 12 hours.
[b]10% mated twice within 24 hours.

Lifetime successful matings

Data obtained on frequency of mating for each sex during a lifetime when (i) caged with a single mate in a 12-dram vial (determined for fed and unfed moths), and (ii) caged with many mates in a 0.21-liter food cup, are given in Table 6, and Fig. 13.

Table 6.--Lifetime matings of fed and unfed potato tuberworm moths caged as individual pairs. N = 40 pairs for each feeding condition.

Number of matings	% Pairs tested	
	Fed	Unfed
0	12.5	7.5
1	22.5	67.5
2	42.5	20.0
3	15.0	5.0
4	5.0	0
5	2.5	0

$\bar{x}$ for fed pairs = 1.9 ± 0.2 range 0 - 5
$\bar{x}$ for unfed pairs = 1.2 ± 0.1 range 0 - 3
means different at 5% level of probability ($t = 2.031$ $t_{39}.05 = 2.028$)

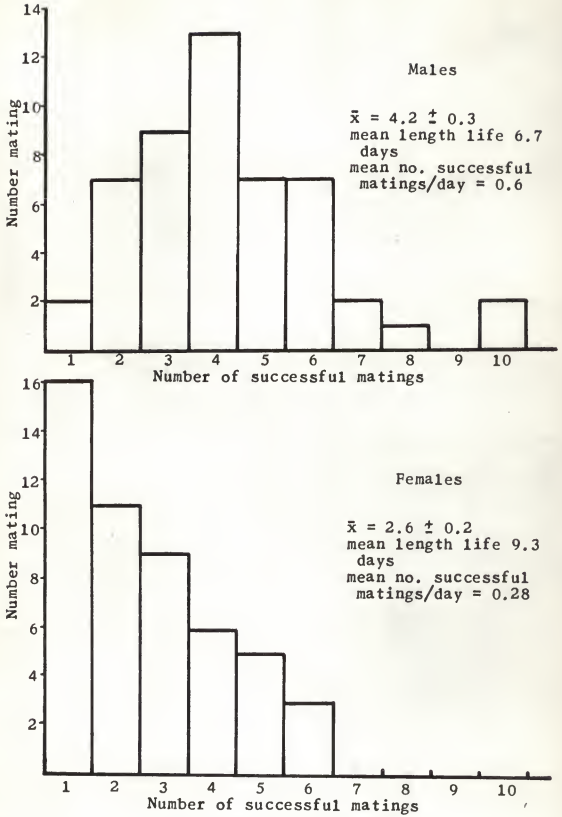


Fig. 13.--Frequency distribution of lifetime successful matings of potato tuberworm moths, caged in 0.21-liter container with continuous supply of virgins of opposite sex. Kept in continuous dark; 10% sucrose solution provided. N = 50 for each sex.

There was a marked contrast between the number of times mating occurred among individual pairs and that which occurred when a continuous fresh supply of virgin moths was provided for the test insect. Mating figures achieved under the latter arrangement are not likely to be duplicated in nature but are a measure of potential successful matings.

The highest mating frequency for males was obtained in an experiment in which 1-day-old virgin males (N = 20) were caged for their lifetime with two 2-day-old virgin females (replaced daily) in a 12-dram vial. The mean number of matings was 7.4 ± 0.4, the mode 7, the range 5 to 11, and the mean number of matings per male per day was 0.9. Fifty per cent of these males mated twice in a single day. These results show that male tuberworm moths are capable of mating virtually every day for as long as they live.

No information is available on mating frequency of field populations of potato tuberworm moths. Ouye, Garcia, Graham, and Martin (1965) reported a mean of 4.2 matings for male pink bollworm moths (N = 220), and 2.3 matings for females (N = 266) when caged with a continuous supply of receptive mates. Field collected females of this species were found to have mated on an average of only 1.1 times (Graham et al., 1965).

Male tuberworm moths caged in 0.21-liter cups, and provided with a fresh supply of virgin females at regular intervals, did not always mate with the virgins. Twenty of 50 males evaluated mated two or three times, or in one case

five times, with the same female, while other females in the same cage remained unmated. A similar situation was observed in the tests in the 12-dram vials where some males mated twice with the same female in a 24-hour period, while the other female remained unmated.

Dustan (1964) suggested that males of the oriental fruit moth had a preference for unmated females. Tuberworm males appear less discriminatory.

Feeding had a significant effect upon mating frequency among single pairs. This difference resulted from fewer multiple matings among unfed moths and not from failure of unfed moths to mate a single time.

Influence of photoperiod on mating and fecundity

Under field conditions, the tuberworm moth is crepuscular and nocturnal in habit, with mating and oviposition occurring during periods of darkness or low light intensity (Graf, 1917; Poos and Peters, 1927). Finney et al. (1947) advocated holding moths in total darkness to encourage mating and improve fecundity, but I have found nothing in the literature to indicate specific effects of photoperiod on these aspects of the behavior of this species.

Tables 7 and 8 summarize the results of experiments conducted to determine the effect of photoperiod on mating and fecundity of tuberworm moths.

There was no significant difference among treatments in terms of influence on mating, fecundity, and spermatophore production. An analysis of variance was run

Table 7.--Influence of photoperiod on mating and fecundity of potato tuberworm moths caged as individual pairs. Based upon four replicates, each of 10 pairs of moths.

Treatment	% Mated♀		No. eggs/mated♀		Spermatophores/♂		% Multiple matings
	Mean	Range	Mean	Range	Mean	Range	
Cont. light	85	80-100	46.7	40.4-51.3	0.95	0.8-1.2	10.0
Cont. dark	72.5	60- 90	43.5	26.5-60.5	0.83	0.7-1.1	12.5
12 hrs light, 12 hrs dark	77.5	60- 90	40.0	30.4-58.7	0.93	0.7-1.1	15.0
1 hr light, 1 hr dark	75.0	70- 80	43.8	27.1-62.7	0.85	0.7-1.0	10.0

Table 8.--Influence of photoperiod on egg production of potato tuberworm moths; 10 pairs per cage.

Treatment	Eggs per replicate			Mean ± SE
	1	2	3	
Continuous light	41.2	39.0	69.9	50.0 ± 2.9
Continuous dark	49.6	60.9	31.6	47.4 ± 2.7

on the data in column 1 and column 2 of Table 7, with F = 0.2 being obtained for the percentage of mated females, and F = 0.9 in the case of the number of eggs per mated female ($F^3_{12}.05 = 3.49$).

Treatment means in Table 8 do not differ significantly, again indicating that photoperiod had no measurable influence on the number of eggs produced by tuberworms caged in groups of 10 pairs.

Despite the absence of statistically significant differences among treatment means, the highest percentage of successful mating occurred under continuous light, and the greatest number of eggs per mated female was also recorded under this light regime.

Whether female moths were caged individually with a male or in groups of 10 pairs per cage seemed to have little effect upon fecundity. It is not possible to compare these data critically, however, because in Table 7 egg production is expressed in terms of number per mated female, whereas in Table 8 it is expressed simply as number per female.

While there was no significant difference between mating frequency in groups of moths kept for 7 days under continuous light and those kept in continuous dark, there was a significant delay in the onset of copulation. Mating frequency determined after 24 hours showed that an average of 92.8 per cent mated within 24 hours of pairing when kept in continuous darkness compared with 60 per cent under continuous light. This difference is significant at the 1 per

cent level of probability ($F = 46.2$ $F^{1}_{6}01 - 13.74$). Based upon these results, there is no reason to suppose that mass rearing of potato tuberworms under continuous light would be less successful than a program in which adult moths were kept in darkness or under a normal day/night regime. The delay in mating caused by continuous light would have little importance in mass rearing.

Precopulatory behavior

A male tuberworm moth exposed to a receptive female displays a characteristic behavior pattern. This same response can be elicited by placing the male in a container in which females have been held for several hours, indicating that a sex pheromone, elaborated by the female, is responsible for initiating precopulatory behavior in the male. (The extraction and assay of the sex pheromone is discussed later.) The description of precopulatory behavior which follows is typical of that seen when a 2-day-old male, held in continuous light from eclosion, was introduced into a 12-dram vial holding a 2-day-old virgin female. The male tuberworm moth in typical resting position had its wings held roof-like over the abdomen and antennae directed posteriorly over the wings. A few seconds after entering the vial the male brought its antennae forward until they formed a "v" with an internal angle of approximately 90^{o}, and held them in this position, motionless except for an occasional twitching. The male then ran excitedly around the container, wings vibrating rapidly, searching for the female. On finding her, the male curved

his abdomen downward, and forward, extended the genital claspers, and "struck" at the terminus of the female abdomen in an attempt to copulate. Copulation occasionally resulted from the very first attempt, but more frequently the male made several "strikes" at the female before the aedeagus was inserted in the ductus bursae. In some cases, where the female for some reason was not receptive, the male persisted in his attempts to mate, circling the female and beating his wings rapidly, making copulatory movements time and time again towards the tip of the female abdomen. This behavior often continued for from 3 to 5 minutes, with the female attempting to avoid the male.

As soon as moths enter copula they move to a position with their heads directed away from each other, then remain motionless (unless disturbed) throughout the mating period.

The precopulatory behavior of male moths, caged with females from the time of eclosion, was much less extreme. In such cases mating normally occurred within the first 24 hours of pairing and was usually accomplished unobtrusively, with a minimum of wing beating and copulatory movements by the male. It is probable that males in this situation respond to low levels of freshly released sex pheromone of a receptive female rather than to a pheromone residue which may bear no relationship to receptivity of the female.

Many female Lepidoptera assume a "calling" position when they are ready to mate. Females in this position norm-

ally extrude the three terminal abdominal segments by curving the abdomen upward and forward over the wings (Roth and Willis, 1952). This action presumably exposes the glands which secrete sex pheromones (Götz, 1951). Some females accompany this extension of the terminal abdominal segments with wing beating (Shorey, 1964). No obvious "calling" behavior was observed with female tuberworm moths. Receptive females made no attempt to avoid the copulatory movements of males, but appeared to be passive partners.

Extraction and Bioassay of Female Sex Pheromone Extraction

Observations of the behavior of male tuberworm moths strongly suggested that the sequence of activities which precede mating was triggered by a pheromone elaborated by the female. It is generally agreed that sex pheromones in Lepidoptera are produced only in glands located at the posterior of the abdomen (Götz, 1951). Extraction of whole female abdomens (Berger et al., 1964) and tips of abdomens (Shorey, Gaston, and Fukuto, 1964) have been used with equal success in sex pheromone studies. For the sake of convenience, extracts of the sex pheromone of the female tuberworm moth were obtained from whole abdomens, after preliminary tests had shown that the use of only the tips of the abdomens gave no measurable improvement in male response.

An average of 10.6 mg of yellow, oily residue was extracted from three groups of 100 whole abdomens of 2-day-old virgin female moths. There was no detectable odor assoc-

iated with the extract, but male moths exposed to the residue responded with typical precopulatory behavior. Extracts of the remainder of the body of the moths did not elicit a response in males.

Bioassay

The response of 60 2-day-old virgin male moths (12 replicates of five moths) to a graded series of concentrations of a crude sex pheromone attractant, expressed in female equivalents, is given in Fig. 14. The response: concentration data were plotted on 4-cycle log probit paper, and the goodness of fit of the eye-fit line was determined, using the Chi-square method as described by Andrewartha (1961).

The lowest concentration of crude attractant to which males responded was 0.25×10^{-3} female equivalents. Ninety-five per cent response was obtained with 0.1 female equivalents. Response to concentrations between these extremes was somewhat erratic, and it was only through the use of 12 replicates that a reasonable curve was obtained.

Shorey and Gaston (1964) showed that light intensity at the time of testing, age of males, time of day, and previous exposure to sex pheromone all had the ability to influence response of male cabbage loopers to female sex pheromone. They felt that the main source of variation in their bioassay results was attributable to contamination of the laboratory air by the female pheromone. An elaborate bioassay apparatus was designed and built by Gaston and

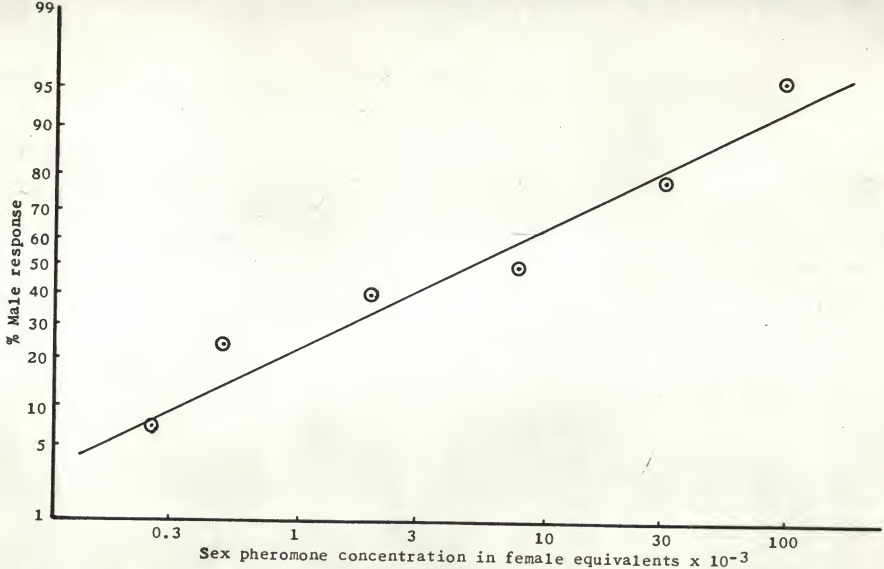


Fig. 14.--Response of 2-day-old virgin potato tuberworm males to female sex pheromone. Sixty males tested per concentration.

Shorey (1964) to overcome this problem of contamination.

In my studies with the tuberworm, assay procedures were standardized insofar as possible, but the possibility of contaminated air in the laboratory, and repression of response due to prior exposure cannot be excluded, and may have contributed to variability in results.

The behavior of males exposed to extracted crude sex pheromone was identical to that described previously for precopulatory behavior. At low concentrations of the pheromone, there was a lag of 15 to 20 seconds between the time the assay cage was placed over the pheromone source and the time that a response was observed in the males. The higher the concentration of pheromone the shorter was this lag period. At concentrations of 0.1 female equivalents, a response was usually seen within 3 seconds to 5 seconds. Males responding to the pheromone raced about the cage with wings vibrating, attempting to copulate with other males. The searching procedure of the males did not always lead them to the bottom of the container, the source of the pheromone and the direction of the air flow. Frequently, a stimulated male would do all of its searching at the top half of the container. The response appeared to be more a case of excitation than attraction--a finding which was consistent with the pattern observed in precopulatory behavior in which a live female provided the sex pheromone.

Influence of age on pheromone content of female

Bioassays were run on extracts from 100 female

abdomens removed from moths: (i) less than 10 minutes old; (ii) 2 days old; and (iii) 8 days old. Females in the latter two categories were kept in continuous light and fed 10 per cent sucrose solution from the time of eclosion until the time of extraction. Five replicates, each of five males, were used at each concentration evaluated. The results are presented graphically in Fig. 15. A 10-to 20-fold increase was required in the concentration of crude pheromone from the 10-minute-old females (Fig. 15C) in order to duplicate the response elicited by the extract from 2-day-old females (Fig. 15A). There was very little difference between the response to extracts from 2-day-old and 8-day-old females.

Tuberworm females did not mate within 3 hours of eclosion, and fewer than 50 per cent of the females evaluated mated within 9 hours of eclosion (Table 5). It is not surprising, therefore, that the sex pheromone content of very young females is less than in 2-day-old virgins. Eight-day-old virgin females are sexually active, although under normal circumstances females have mated and laid all of their eggs by this age. Shorey and Gaston (1965) found that sex pheromone content of female cabbage loopers increased from the time of emergence up until the second day, after which the quantity stabilized. Females of cabbage loopers seldom mate until 2 days old, but remain sexually active for most of their life (Shorey, 1964).

Extinction of male response to sex pheromone

Groups of males exposed to high concentrations of

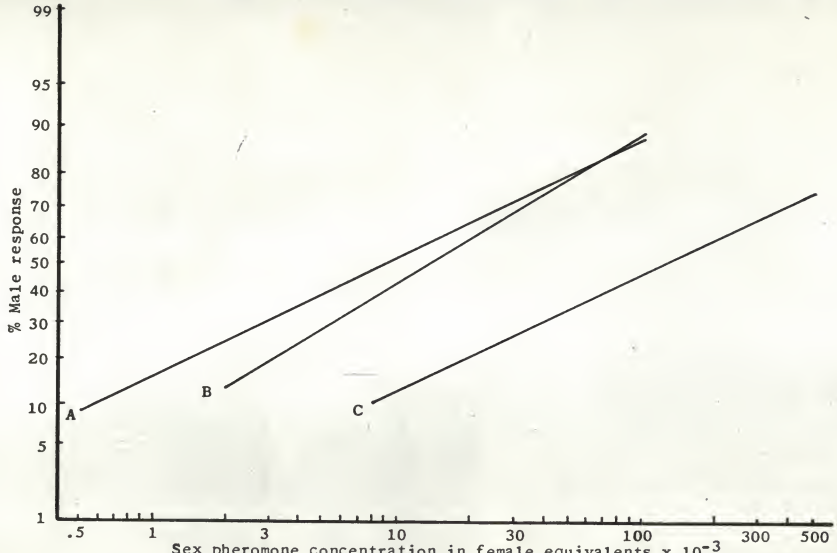


Fig. 15.--Influence of age of females, at time of extraction of sex pheromone, on response of 2-day-old potato tuberworm males. Based on five replicates each with five males. Ages of females: A less than 10 minutes; B 2 days; C 8 days.

the crude sex pheromone in the bioassay apparatus responded very excitedly by whirling around the cage, vibrating their wings rapidly, and attempting to copulate with other males in the cage. This behavior normally persisted with little or no interruption for 2 to 4 minutes. In one instance, five males in an assay cage kept up this excited response for 8 minutes. A period of quiet, usually 1 or 2 minutes long, was followed by short bursts of activity of 20 to 30 seconds duration. These responses became less frequent and less prolonged, and males were unresponsive after 6 to 8 minutes. In several tests, males continued to show bursts of copulatory activity for 15 minutes, and in one instance, activity persisted for 40 minutes among a group of five males. Males in which the response to female sex pheromone had become extinct were held under an exhaust hood and then re-exposed to the pheromone after varying periods of time. A full response was normally achieved after a recovery period of 10 minutes, but the response was less prolonged than in the first exposure. Shorey (1964) reported that copulatory movements of males of Trichoplusia ni, exposed to 0.1 female equivalents of sex pheromone, generally ceased after about 1 minute. He suggested that this represented extinction of the response due to sensory adaption, and that such a mechanism would have survival benefit, since males would not expend energy unnecessarily when exposed to the sex pheromone of an inaccessible female. The relatively long period of response observed in the tuberworm may be necessary to com-

pensate for what appears to be a low order of attraction associated with the female sex pheromone in this species.

Site of reception of sex pheromone

There are a great many references in the literature establishing the antennae of insects as the principal site of olfactory receptors (Dethier, 1953; Schneider, 1964).

When antennae were removed, male tuberworm moths subjected to the bioassay procedure showed no response to high levels of sex pheromone. No mating took place between 20 pairs of moths caged in 12-dram vials when antennae were removed from the males.

Trapping males with female sex pheromone

A summary of catches made in traps baited with 20 female equivalents is given in Table 9.

Many males displayed the typical precopulatory behavior when first introduced into the cage in which the baited and control traps were set. Most of the activity took place on the screen sides of the cage, downwind from the trap. Searching was principally carried on by running over the screen rather than by flying. All moths which were seen to enter the baited trap did so by flying against the air flow, leaving the side of the cage at a point almost directly in line with the trap opening, and flying in a fairly straight line to the bait. Males in such cases were attracted to the trap from a distance of approximately 0.61 m.

Kettlewell (1946) suggested that male moths fly to females only upwind and the studies of Schwinck (1958) and

Shorey (1964) have supported this now generally accepted theory. The concentration gradient of sex pheromones apparently is not the orientation factor at long distances, but the pheromone serves only to trigger a sequence of events typified by random, searching flight, leading to orientation against the wind when the female odor is detected, and upwind flight by the male (Schwinck, 1958).

Table 9.--Number of 2-day old virgin male tuberworm moths caught in 24 hours in trap baited with 20 female equivalents of crude female sex pheromone set in cage 1.2 m x 1.2 m x 1.8 m (high). Fifty males/replicate.

Replicate	Number of moths trapped			
	Light regime			Total in 24 hrs
	1 hr light	12 hrs dark	11 hrs light	
1	4	0	3	7
2	3	2	5	10
3	2	0	2	4
4	6	4	4	14
mean	3.75	1.50	3.50	8.75 = 17.5%
Control[a]	0	0	.25	.25 = .5%

[a]average of four replicates

Twenty-nine of the total of 35 male tuberworm moths trapped in these tests were caught during periods of light (6 ft-c intensity). Observations made of moths during the dark periods (using a heavily hooded light) showed very little activity. It must be remembered, however, that male moths used in these tests had been held in continuous light since eclosion. Graf (1917), and Poos and Peters (1927) reported tuberworm moths to be most active sexually in the

field during the hours of twilight, particularly the evening. However, three trapping tests that I carried out under conditions of natural light in a cage 0.61 m x 0.61 m x 0.61 m using 10 live 2-day-old virgin females as the attractant, failed to give a significant catch (over the unbaited control) of 2-day-old virgin males.

Tests (three replicates) in which a trap was baited with 50 female equivalents and set 1.2 m above the floor in the center of a room 4.6 m x 3.4 m x 2.4 m (high) failed, in 24 hours, to attract and catch any males out of an estimated population of 500 moths of mixed age and sex. These tests were run under conditions of natural light.

The results of the trapping experiments reinforce the view that the pheromone elaborated by the female tuberworm moth, or at least the crude extract of this pheromone, is primarily an excitant rather than an attractant. Schwinck (1958) showed that odor attraction of the male silkworm moths by the female could only be demonstrated for close orientation. At greater distances the pheromone had only an excitory effect, eliciting a searching behavior in the male.

In evaluating the importance of the pheromone it is necessary to consider the habits of the species in nature. The tuberworm is basically a weak flyer, seldom moving more than a few feet in a single flight when disturbed in the field (Hofmaster, 1949). Under conditions of storage, where the pest is of particular importance, moths usually travel by walking rather than by flying. Considering these circum-

stances, failure of the female sex pheromone to attract males over long distances is not too surprising. The possibility of using the sex pheromone of the female potato tuberworm moth in a control program is not encouraging.

Effect of Metepa on Potato Tuberworm Moths

Preliminary tests

A series of tests was carried out to determine: (i) a satisfactory way to immobilize moths during treatment; and (ii) the effect of acetone, the intended solvent for metepa, on longevity and mating behavior.

Carbon dioxide, ethyl ether, and low temperature were evaluated as methods of immobilizing moths. Ether proved most satisfactory, being more convenient than low temperature and having a less critical exposure time than carbon dioxide. Moths lightly anesthetized with ether remained subdued for several minutes, whereas those anesthetized with carbon dioxide revived so rapidly that post-treatment handling was made difficult.

One microliter of reagent grade acetone applied topically to the thoracic sternum of anesthetized moths caused no measurable effect on longevity or mating behavior.

In initial tests, moths were treated and then caged immediately with untreated 2-day-old virgin moths of the opposite sex. Frequently mating occurred within a matter of seconds and the eggs produced in these cases showed a higher viability than those from moths which had been similarly treated but which had not mated as quickly.

To avoid any possibility of fertilization of eggs taking place before the chemosterilant had an opportunity to take effect, treated moths were held for 1 day and then paired with 2-day-old virgin mates.

Chemosterilization studies

Results obtained from tests in which 1-day old virgin moths were treated with metepa, applied topically to the thoracic sternum, are given in Tables 10 and 11 and Figs. 16 and 17. Dosage: response data were plotted on log probit paper and the goodness of fit of the eye-fit line verified by a Chi-square test (Andrewartha, 1961).

Males.--Ninety-nine per cent sterility was achieved in male tuberworm moths with a dose of 15.0 μg of metepa (Table 10), but this was accompanied by a high level of mortality among treated moths (Table 10, and Fig. 16). An ED_{50} (sterility) of 8 μg was recorded and an LD_{50} of 15.5μg (Fig. 16). Chang and Borkovec (1966) devised a simple method of expressing the relationship between sterility and mortality in a way that would serve as an index of the safety of the chemosterilant at the level of complete sterilization. They derived the index by determining the difference between the maximum dose which resulted in no mortality and the minimum dose which caused complete sterility, the difference being divided by the latter. The index was termed a "safety factor." A positive value indicates theoretical feasibility of sterilization of the species, while a negative value means that complete sterility cannot be achieved without

Table 10.--Effect of metepa on mortality, mating, fecundity (of mate), and egg hatch when applied topically to the thoracic sternum of 1-day-old virgin male potato tuberworm moths. N = 40 for each concentration. Moths caged with untreated females as individual pairs.

Dosage in µg/moth	% Mortality at 48 hrs	Mating	Eggs Total	Mean per mated female	Mean % hatch
5.0	0	97.5	2601	66.7	87.5
7.5	2.5	97.5	2127	54.6	50.1
10.0	20.0	67.5	1138	42.2	24.7
12.5	30.0	60.0	987	38.0	18.5
15.0	47.5	52.5	1092	52.0	0.7
17.5	60.0	47.5	821	43.2	0.25
20.0	75.0	42.5	882	51.9	0.5
22.5	77.5	25.0	348	34.8	0
25.0	95.0	20.0	376	47.0	0
30.0	100.0	25.0	493	49.3	0
Control	0	95.0	2078	54.7	92.0

Table 11.--Effect of metepa on mortality, mating, fecundity, and egg hatch, when applied topically to the thoracic sternum of 1-day-old virgin female potato tuberworm moths. N = 40 for each concentration. Moths caged with untreated males as individual pairs.

Dosage in µg/moth	% Mortality at 48 hrs	Mating	Eggs Total	Mean per mated female	Mean % hatch
10.0	0	90.0	1750	48.5	74.3
12.5	7.5	85.0	1367	40.2	69.1
15.0	10.0	72.5	1188	41.0	55.7
17.5	22.5	87.5	812	23.2	23.3
20.0	47.5	57.5	816	35.4	21.9
22.5	55.0	50.0	455	22.8	31.8
25.0	80.0	47.5	390	20.5	16.7
27.5	87.5	20.0	173	21.6	3.5
30.0	87.5	20.0	124	15.5	5.6
32.5	95.0	27.5	124	11.3	6.0
40.0	100.0	25.0	60	6.0	10.0
Control	0	92.5	1719	46.4	90.8

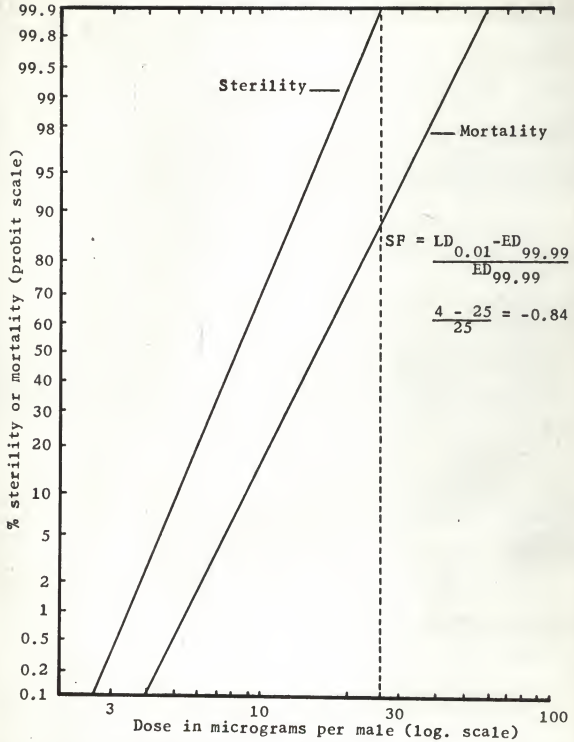


Fig. 16.--Relationship between sterility and mortality in 2-day-old male potato tuberworm moths treated with metepa applied topically on the thoracic sternum. Data corrected by Abbott's formula.

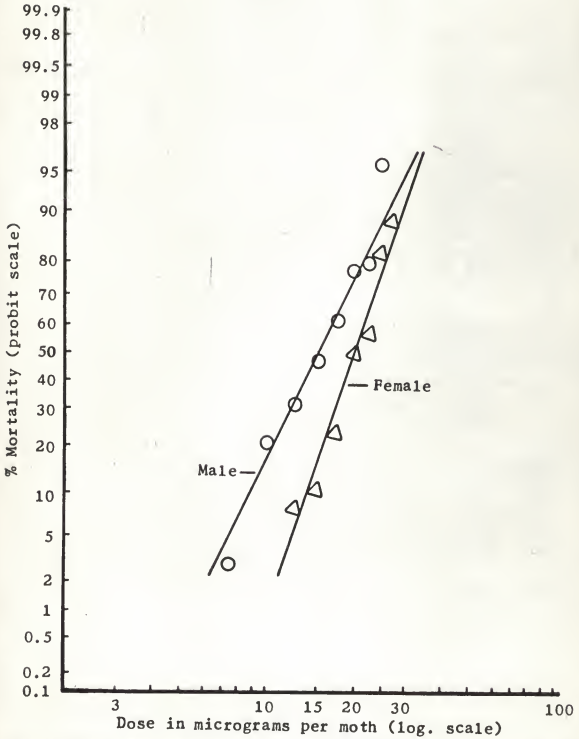


Fig. 17.--Dosage:mortality curves for male and female potato tuberworm moths treated topically with metepa.

sacrificing a portion of the treated sample. I obtained a safety factor of -0.84 for metepa on the male tuberworm moth, confirming the unsatisfactory relationship between sterility and mortality when this compound is applied topically (Fig. 16).

The number of eggs produced per mated female at the various dosages could not be compared statistically because of wide variation in the number of males mating at the different concentrations. However, there was no indication of reduced egg production in females mated to males receiving high doses of metepa. Similarly, excessive mortality prevented a statistical evaluation of the effect of metepa on frequency of mating. At high dosages some males that ultimately died within 48 hours were able to mate. Males treated with 7.5μg did not show any reduction in mating frequency from the control, but approximately 50 per cent sterility resulted from the treatment. Ouye, Graham, et al. (1965) reported a slight repression of mating frequency in pink bollworm moths sterilized with metepa, and Henneberry, Shorey and Kishaba (1966) found a similar repression in cabbage looper males sprayed with 1 per cent tepa.

A few eggs hatched from pairing of females with males treated with 17.5 μg and 20.0 μg of metepa. It is possible that variations in application technique, in penetration of the cuticle by the sterilant, or even parthenogenetic reproduction accounted for this difficulty in getting absolute sterility once the 99 per cent level had been

obtained. Linkfield (1966) was unable to satisfactorily explain failure to get complete sterility in males of the oriental rat flea, Xenopsylla cheopis (Rothschild), treated with tepa.

Female.--A comparison of the dosage: response curves for male and female tuberworm moths is given in Fig. 17. The LD_{50} for the female moths was 20 μg, whereas that for males was 15.5 μg. Complete sterility of the female was not achieved. Female Lepidoptera are generally more difficult to sterilize than males (Collier and Downey, 1965; Young and Cox, 1965). The differences in LD_{50} for male and female tuberworm moths may very well be a reflection of differences in weight of the sexes. One-day-old virgin females were found to weigh 8.1 $\pm$ 0.23 mg, compared to 5.2 $\pm$ 0.2 mg for the males (N = 50). If the lethal dose is expressed in micrograms of metepa per milligrams of insect body weight the LD_{50} for females is 2.48 μg and for the males 2.95 μg.

Female insects treated with chemosterilants typically produce fewer eggs than untreated females. This tendency was taken into account by Chamberlain (1962), and Crystal (1963) in drawing up formulae to express reduction in reproduction attributable to chemosterilants. High mortality among tuberworm females in the experiments reported here prevented statistical evaluation of the effect of metepa on fecundity, but there is an apparent suppression of egg laying at the high dosages of metepa (Table 11).

Chemosterilization of Lepidoptera generally has

been erratic (Collier and Downey, 1965; Young and Cox, 1965; Henneberry and Kishaba, 1966). However, Ouye, Garcia, and Martin (1965) were able to sterilize males of the cotton bollworm with 15 μg of metepa applied topically, and reported only 2 per cent mortality at 48 hours among males treated with 50 μg. Males treated with 15 μg metepa were completely competitive with untreated males in mating with females (Ouye, Graham et al. 1965). Bulyginskaya (1965) sterilized the hollyhock seed moth, Pectinophora malvella Hubbard with 0.1 per cent thio-tepa fed in a sugar solution, with little mortality or effect on longevity. Both of the above species are in the same family as the potato tuberworm (Gelechiidae).

The toxic action of aziridinyl compounds on insect systems, other than the reproductive system, is not understood. Alexander (1960) stressed that alkylation may occur at many sites within the insect and that these sites are not restricted to the reproductive system. This is born out by the adverse effects that alkylating agents often have on longevity and mating of treated insects, as evidenced in this study with the tuberworm. Some alternative method of application of metepa, or perhaps some other alkylating agent might yet provide a means of successfully sterilizing the potato tuberworm moth. I hope to investigate this possibility at some later date.

SUMMARY

The possibility of eradicating the potato tuberworm from the island of Bermuda through a program of chemosterilization prompted this study.

The reproductive systems of male and female moths are illustrated and discussed, including the form of the spermatophore and its placement in the female. Comparisons are made with other Lepidoptera. The presence of a spermatophore in the bursa copulatrix was used throughout the study as the criterion for successful mating.

Tuberworm moths were capable of mating within 6 hours from the time of eclosion and 95 per cent of the moths tested mated within 24 hours of eclosion when held in continuous darkness. The average time required for successful mating was 100.3 minutes (N = 116), with the minimum time being 43 minutes. In no instance was more than one spermatophore passed during a single pairing. Males mated an average of 4.2 times and females 2.6 times (N = 50) during a lifetime when provided with a continuous supply of virgin partners. Moths caged as individual pairs mated 1.2 times during a lifetime when unfed, and 1.9 times when fed (N = 40).

Parthenogenetic reproduction was observed in 1.5 per cent of 200 females tested, but this in itself is not considered a serious handicap to control of the species by autocidal methods.

There was no significant difference in frequency of mating and fecundity among moths held under four different light regimes when evaluated after 8 days. However, continuous light, in comparison to continuous dark, did reduce the mating frequency during the first 24 hours of pairing.

The presence of a female sex pheromone was established and extraction accomplished from whole abdomens using methylene chloride as the solvent. Bioassay procedures and trapping tests showed the pheromone to be primarily an excitant rather than an attractant. The pheromone elicited a response in 2-day-old virgin males at a concentration of 0.25×10^{-3} female equivalents. Crude extract of pheromone from freshly emerged females was 10 to 20 times less potent than that from 2-day-old virgins.

The chemosterilant metepa, applied topically in acetone, sterilized 99 per cent of the male moths treated at a dose of 15 μg per moth, but this same dose caused approximately 50 per cent mortality at 48 hours. Sterilization of the female was erratic and was also associated with high mortality. The LD_{50} for male moths, expressed as μg/moth, was 15.5 μg, whereas that for females was 20.0 μg. If the lethal dose is expressed in micrograms of metepa per milligram of insect body weight the LD_{50} for males is 2.98 μg and that of the females 2.48 μg. The results indicate that metepa is unsuited for sterilization of the potato tuberworm moth.

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BIOGRAPHICAL SKETCH

Idwal Wyn Hughes was born in Hamilton, Bermuda, on February 21, 1932. He received his primary and secondary education at the Whitney Institute, Bermuda, graduating in February, 1949. After spending one year at Mount Allison Academy in Sackville, New Brunswick, Canada, he entered Cornell University in the fall of 1950, and received the degree of Bachelor of Science in Agriculture (Entomology) in June, 1954. He was appointed to the post of Plant Pathologist with the Bermuda Department of Agriculture on July 1, 1954, and on January 1, 1956, to that of Assistant Director of Agriculture, a position he now holds.

The writer was admitted to the Graduate School of the University of Florida in February, 1959, and received the degree of Master of Science in Agriculture in February, 1960. He returned to the University of Florida in September, 1965, on a graduate assistantship, to work toward the degree of Doctor of Philosophy.

Idwal Wyn Hughes is married to the former Catherine Elizabeth King, and is the father of three children. He is a member of the Society of Sigma Xi; Phi Kappa Phi; Gamma Sigma Delta; Entomological Society of America; and the Florida Entomological Society.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April 22, 1967.

Dean, College of Agriculture

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